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USE OF MICROCULTURE PLATES AND THE MULTIPLE AUTOMATED SAMPLE HARVESTER FOR IN VITRO MICROASSAY OF BACTERIAL TOXINS

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Use of Microculture Plates and the Multiple Automated Sample Harvester for In Vitro Microassay of Bacterial Toxins

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An in vitro cytotoxicity microassay for the measurement of nanogram quantities of *Pseudomonas aeruginosa* exotoxin and *Vibrio cholerae* enterotoxin is described.

An in vitro microassay for lymphotoxin using mouse L-929 fibroblasts (L cells) grown in wells of microculture plates has recently been described (5). Test material is added to the culture medium, and cytotoxic activity is measured as the reduction in incorporation of [*methyl*-^{3}H]thymidine by target cells. Because this assay is sensitive and versatile, and because it requires a minimal amount of time and materials, we thought it might prove useful in studying other naturally occurring toxins known to have cytotoxic properties. Our study illustrates the use of this microassay procedure in measuring cytotoxic activity of two bacterial protein exotoxins: *Pseudomonas aeruginosa* exotoxin (PE), which has been shown to be toxic for cultivated Vero cells (6); and *Vibrio cholerae* enterotoxin (CE), which has been shown to be cytotoxic for mouse spleen cells (7) and adrenal cells (4).

The purified cholera toxin (a gift from Stephen H. Richardson, Bowman Gray School of Medicine, Winston-Salem, N.C.) had a specific activity of 7,000 to 10,000 blueing doses per μg of protein, as measured by the skin permeability bioassay in rabbits (3), and contained 440 μg of Lowry protein per ml. PE, purified as described previously (2), contained 350 μg of Lowry protein per ml and had a mean lethal dose of 4 μg when assayed in mice weighing 20 ± 2 g.

The microassay has been described in detail elsewhere (5). For comparative purposes, both L cells and HeLa cells were used as target cells in this experiment. Approximately 1,000 HeLa cells or L cells, in 50 μliters of RPMI 1640 plus supplements (5), was dispensed into each well of several microculture plates. Fifty microliters of twofold serial dilutions of each toxin preparation was subsequently added to the microcultures. Each dilution was dispensed into triplicate cultures. An equivalent amount of medium was dispensed into three microcultures to serve as controls for normal cell growth. Cultures were incubated for 4 days. Twenty-four hours prior to harvesting, 20 μliters of medium containing 1 μCi of [*methyl*-^{3}H]thymidine (specific activity 1.9 Ci/mmol) was added to each microculture. Target cells were harvested from microculture wells onto glass-fiber filter disks using the multiple automated sample harvester (1). Mean counts per minute of triplicate cultures were converted into percent inhibition using the formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{mean counts per minute of toxin wells}}{\text{mean counts per minute of control wells}} \times 100$$

The sensitivity of the microassay in measuring PE and CE activity for HeLa cells and L cells is shown in Fig. 1. A 50% inhibition of L cell growth required only 1 ng of PE and 81.5 ng of CE. In contrast, 50% inhibition of HeLa cell growth required 145 ng of PE and 352 ng of CE.

Microscope observations of microcultures showing 99% inhibition of [*methyl*-^{3}H]thymidine incorporation revealed that virtually all target cells were destroyed. Complete destruction of L cells required 27 ng of PE and 4,400 ng of CE. Thus, both complete destruction and 50% inhibition of target cell growth were more sensitive to PE than CE, and L cells were far more sensitive to both PE and CE than HeLa cells. In contrast to these results, *Escherichia coli* endotoxin (Difco Laboratories, Detroit, Mich.) has been found not to affect the growth of L cells at concentrations of 10 μg per microculture.

These results demonstrate that the microas-

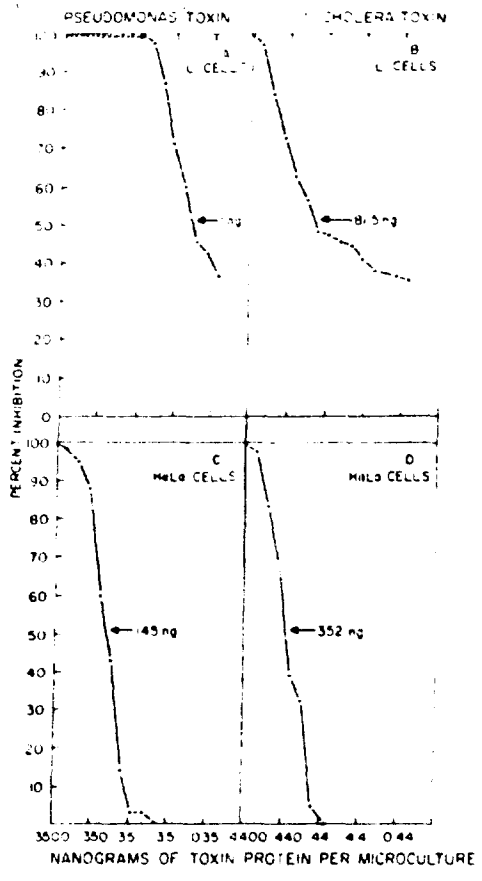

FIG. 1. *Inhibition of L cell and HeLa cell growth by dilutions of Pseudomonas and Cholera toxin. Mean counts per minute (± standard error) for control microcultures were: (A) 31,060 ± 1,167; (B) 34,409 ± 1,106; (C) 24,512 ± 1,755; and (D) 21,951 ± 1,088. The mean inhibitory dose = dilution which inhibits target cell growth by 50%*

say is sensitive and requires small amounts of materials, and that large numbers of assays can readily be performed. Only a few minutes are required to harvest a microculture plate using the multiple automated sample harvester. As many as 30 microculture plates have been assayed at one time in this laboratory. The procedure should be adaptable to different target cells, radioactive labels, and incubation periods, and should prove useful in evaluating the effectiveness of antisera and chemicals in blocking toxic activity of various toxins.

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